

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1. (currently amended) A method of performing high throughput mass spectrometry screening, the method comprising:
 - (i) providing one or more cell comprising a gene variant library,
 - (ii) growing the one or more cell in vitro thereby providing non-column separated components comprising a library of gene expression products;
 - (iii) purifying samples comprising the non-column-separated components with an off-line parallel purification system,
wherein the non-column-separated components have not undergone prior separation on a chromatography column and wherein the purifying step is not a chromatographic separation step;
 - (iv) injecting the purified samples generated from step (iii) into a mass spectrometer; and
 - (v) performing flow-injection analysis using electrospray tandem mass spectrometry on the purified samples to detect the presence of one or more component of interest, thereby obtaining mass-to-charge ratio data and providing high throughput mass spectrometry screening of the one or more component of interest,
wherein the one or more component of interest is from the one or more cell.
2. (previously presented) The method of claim 1, wherein step (ii) occurs simultaneously with step (iii), and wherein said one or more cell is alive during step (iii).
3. (previously presented) The method of claims 1 or 2, wherein at least about 100 samples are screened for presence of the one or more component of interest in less than an hour.

4. (previously presented) The method of claim 1, wherein at least about 200 samples are screened for presence of the one or more non-column-separated component of interest in less than an hour.

5. (previously presented) The method of claim 1, wherein at least about 500 non-column-separated samples are screened for presence of the one or more component of interest in less than an hour.

6. (previously presented) The method of claim 1, wherein at least about 1000 samples are screened for the presence of the one or more component of interest in about 1 day.

Claims 7-11 (canceled)

12. (previously presented) The method of claim 1, wherein said purifying one or more non-column-separated samples comprises performing step (iii) in a volatile buffer, a buffer that reduces concentration of ionic species, an ion exchange resin, or an organic solvent.

13. (previously presented) The method of claim 1, wherein the non-column-separated samples comprise cell lysate.

14. (previously presented) The method of claim 1, wherein the one or more component of interest is selected from the group consisting of a protein, a protein binding molecule, a carbohydrate, a carbohydrate binding molecule, a product of an enzyme catalyzed reaction, a nucleic acid, and a product of a nucleic acid catalyzed reaction.

15. (previously presented) The method of claim 1, wherein the one or more component of interest is selected from: an enzyme, an enzyme substrate, and an enzyme product.

16. (previously presented) The method of claim 1, wherein the component of interest is selected from: a substrate with one or more hydrophobic moieties, an inorganic ion, an oligosaccharide, a hydrophobic molecule, atrazine, and a polyketide.

17. (previously presented) The method of claim 1, wherein purifying the non-column-separated samples comprises attaching the library of expression products to a solid support.

18. (canceled)

19. (previously presented) The method of claim 17, wherein the library of expression products comprises a library of enzymes, which enzymes each comprises a tag moiety, and wherein the solid support comprises a tag binding moiety.

20 (Original) The method of claim 19, wherein the tag moiety comprises biotin, avidin, or streptavidin and the tag binding moiety comprises biotin, avidin, or streptavidin.

21. (canceled)

22. (previously presented) The method of claim 1, wherein the one or more component of interest comprise enzyme substrate and product of an enzymatic reaction, the method further comprising simultaneously quantifying the amount of the product of an enzyme reaction and the enzyme substrate.

23. (previously presented) The method of claim 1, wherein performing flow injection analysis using electrospray tandem mass spectrometry comprises performing a method selected from the group consisting of neutral loss mass spectrometry and parent ion mass spectrometry.

24. (Original) The method of claim 23, comprising performing the neutral loss mass spectrometry or the parent ion mass spectrometry on a triple quadrupole mass spectrometer.

25. (previously presented) The method of claim 24, wherein performing the neutral loss mass spectrometry comprises:

- (a) scanning the one or more component of interest in a first quadrupole at a specified mass range;
- (b) fragmenting the one or more component of interest in a second quadrupole by collision induced dissociation, thereby producing one or more neutral fragments and one or more daughter ion; and,
- (c) detecting the one or more daughter ion.

26. (previously presented) The method of claim 24, wherein performing the parent ion mass spectrometry comprises:

- (a) scanning the one or more component of interest in a first quadrupole;
- (b) fragmenting the one or more component of interest in a second quadrupole by collision induced dissociation; and,
- (c) scanning a third quadrupole at a specified mass.

Claims 27-71 (canceled)

72. (previously presented) The method of claim 1, wherein purifying comprises centrifugation.

73. (previously presented) The method of claim 1, wherein purifying comprises filtration.

74. (previously presented) The method of claim 1, wherein the off-line parallel purification system comprises an ion exchange resin.

75. (previously presented) The method of claim 1, wherein the off-line parallel purification system comprises the addition of an organic solvent to the sample.

76. (previously presented) The method of claim 1, wherein the off-line parallel purification system comprises a solid phase extraction plate.

77. (previously presented) The method of claim 1, wherein an automatic sampler transports samples from the off-line parallel purification system to the mass spectrometer for injection and analysis at a rate of at least 100 samples an hour.

78. (previously presented) The method of claim 1, wherein 5 to 100 non-column separated samples are pooled before performing flow-injection analysis using electrospray tandem mass spectrometry.

Claims 79-80 (canceled)

Claims 81-104 (canceled)

105. (currently amended) A method of performing high throughput mass spectrometry screening, the method comprising:

- (i) providing one or more cell comprising a gene variant library,
wherein the gene variant library encodes an enzyme library;
- (ii) growing the one or more cell in vitro to provide an enzyme library;
- (iii) contacting the enzyme library with an enzyme substrate thereby providing non-column-separated components comprising the enzyme library and product of an enzymatic reaction;
- (iv) purifying samples comprising the non-column-separated components with an off-line parallel purification system,

wherein the non-column-separated components have not undergone prior separation on a chromatography column and wherein the purifying step is not a chromatographic separation step;

- (v) injecting the purified samples into a mass spectrometer; and,
- (vi) performing flow-injection analysis using electrospray tandem mass spectrometry on the purified samples, thereby obtaining mass-to-charge ratio data and providing high throughput mass spectrometry screening for presence of one or more component of interest,

wherein the component of interest is selected from the group consisting of the enzyme substrate, the product of an enzymatic reaction, and an enzyme.

106. (previously presented) The method of claim 105, wherein at least about 100 samples are screened for presence of the one or more component of interest in less than an hour.

107. (previously presented) The method of claim 105, wherein at least about 200 samples are screened for presence of the one or more component of interest in less than an hour.

108. (previously presented) The method of claim 105, wherein at least about 500 samples are screened for presence of the one or more component of interest in less than an hour.

109. (previously presented) The method of claim 105, wherein at least about 1000 samples are screened for the presence of the one or more component of interest in about 1 day.

110. (previously presented) The method of claim 105, wherein the non-column-separated samples comprise cell lysate.

111. (canceled)

112. (previously presented) The method of claim 105, wherein purifying the non-column-separated samples comprises attaching the enzyme library to a solid support.

113. (previously presented) The method of claim 112, wherein each enzyme in the library further comprises a tag moiety, and wherein the solid support comprises a tag binding moiety.

114. (previously presented) The method of claim 113, wherein the tag moiety comprises biotin, avidin, or streptavidin and the tag binding moiety comprises biotin, avidin, or streptavidin.

115. (previously presented) The method of claim 105, wherein the one or more component of interest comprises the enzyme substrate and the product of an enzymatic reaction, the method further comprising simultaneously quantifying the amount of the product of an enzyme reaction and the enzyme substrate.

116. (previously presented) The method of claim 105, wherein performing flow injection analysis using electrospray tandem mass spectrometry comprises performing a method selected from the group consisting of neutral loss mass spectrometry and parent ion mass spectrometry.

117. (previously presented) The method of claim 116, comprising performing the neutral loss mass spectrometry or the parent ion mass spectrometry on a triple quadrupole mass spectrometer.

118 (previously presented) The method of claim 117, wherein performing the neutral loss mass spectrometry comprises:

- (a) scanning the one or more component of interest in a first quadrupole at a specified mass range;
- (b) fragmenting the one or more component of interest in a second quadrupole by collision induced dissociation, thereby producing one or more neutral fragments and one or more daughter ion; and,
- (c) detecting the one or more daughter ion.

119. (previously presented) The method of claim 117, wherein performing the parent ion mass spectrometry comprises:

- (a) scanning the one or more component of interest in a first quadrupole;
- (b) fragmenting the one or more component of interest in a second quadrupole by collision induced dissociation; and,
- (c) scanning a third quadrupole at a specified mass.

120. (previously presented) The method of claim 105, wherein purifying comprises centrifugation.

121. (previously presented) The method of claim 105, wherein purifying comprises filtration.

122. (canceled)

123. (previously presented) The method of claim 105, wherein the off-line parallel purification system comprises the addition of an organic solvent to the sample.

124. (canceled)

125. (previously presented) The method of claim 105, wherein an automatic sampler transports samples from the off-line parallel purification system to the mass spectrometer for injection and analysis at a rate of at least 100 samples an hour.

126. (previously presented) The method of claim 105, wherein 5 to 100 samples are pooled before performing flow-injection analysis using electrospray tandem mass spectrometry.